

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 2 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2001:862522 SCISEARCH
THE GENUINE ARTICLE: 486CN
TITLE: High lipase production by *Candida rugosa* is associated with G1 cells. A flow cytometry study
AUTHOR: Lotti M (Reprint); Brocca S; Porro D
CORPORATE SOURCE: Univ Milan, Dipartimento Biotecnol & Biosci, Piazza Sci 2, I-20126 Milan, Italy (Reprint); Univ Milan, Dipartimento Biotecnol & Biosci, I-20126 Milan, Italy
COUNTRY OF AUTHOR: Italy
SOURCE: BIOTECHNOLOGY LETTERS, (NOV 2001) Vol. 23, No. 21, pp. 1803-1808.
Publisher: KLUWER ACADEMIC PUBL, SPUIBOULEVARD 50, PO BOX 17, 3300 AA DORDRECHT, NETHERLANDS.
ISSN: 0141-5492.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 15

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Growth of *C. rugosa* on three different culture media was analysed by laser flow cytometry to evaluate physiological growth conditions allowing effective lipase production. The highest productivity was associated with an increased proportion of cells in the G1 phase and was independent of the effect of the medium on lipase formation.

L22 ANSWER 3 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 2001:547452 SCISEARCH
THE GENUINE ARTICLE: 447RJ
TITLE: Crystal structure of 2-hydroxyl-6-oxo-6-phenylhexa-2,4-dienoic acid (HPDA) hydrolase (BphD enzyme) from the *Rhodococcus* sp strain RHA1 of the PCB degradation pathway
AUTHOR: Nandhagopal N; Yamada A; Hatta T; Masai E; Fukuda M; Mitsui Y; Senda T (Reprint)
CORPORATE SOURCE: Natl Inst Adv Ind Sci & Technol, Biol Informat Res Ctr, Kohto Ku, Tokyo 1350006, Japan (Reprint); Nagaoka Univ Technol, Dept Bioengn, Div Prot Engn, Niigata 9402188, Japan; Nagaoka Univ Technol, Dept Bioengn, Div Microbial Engn, Niigata 9402188, Japan; Okayama Univ, Res Inst Technol, Okayama 703, Japan
COUNTRY OF AUTHOR: Japan
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (22 JUN 2001) Vol. 309, No. 5, pp. 1139-1151.
Publisher: ACADEMIC PRESS LTD, 24-28 OVAL RD, LONDON NW1 7DX, ENGLAND.
ISSN: 0022-2836.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 50

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB 2-Hydroxyl-6-oxo-6-phenylhexa-2, 2-dienoic acid (HPDA) hydrolase (the BphD enzyme) hydrolyzes a ring-cleavage product of an aromatic compound generated in a biphenyl/polychlorinated biphenyl (PCB) degradation pathway of bacteria. The crystal structure of the BphD enzyme has been determined at 2.4 Angstrom resolution by the multiple isomorphous replacement method. The final refined model of the BphD enzyme yields an R-factor of 17.5% at 2.4 Angstrom resolution with reasonable geometry. The BphD enzyme is an octameric enzyme with a 422 point-group symmetry. The subunit can be divided into core and lid domains. The active site of the enzyme is situated in the substrate-binding pocket, which is located between the two domains. The substrate-binding pocket can be divided into hydrophobic and hydrophilic regions. This feature of the pocket seems to be necessary for substrate binding, as the substrate is composed of hydrophilic and hydrophobic parts. The proposed orientation of the substrate seems to be consistent with the general catalytic mechanism of alpha/beta -hydrolases. (C) 2001 Academic Press.

L22 ANSWER 4 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on
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ACCESSION NUMBER: 2001:955751 SCISEARCH
THE GENUINE ARTICLE: 497MN
TITLE: Three new **lipases** from actinomycetes and their
use in organic reactions
AUTHOR: Cardenas F; Alvarez E; De Castro-Alvarez M S;
Sanchez-Montero J M; Elson S; Sinisterra J V (Reprint)
CORPORATE SOURCE: Univ Complutense Madrid, Fac Pharm, Dept Organ &
Pharmaceut Chem, E-28040 Madrid, Spain (Reprint);
SmithKline Beecham, Ctr Invest Basica, Madrid 28760, Spain
COUNTRY OF AUTHOR: Spain
SOURCE: BIOCATALYSIS AND BIOTRANSFORMATION, (29 NOV 2001) Vol. 19,
No. 4, pp. 315-329.
Publisher: HARWOOD ACAD PUBL GMBH, TAYLOR & FRANCIS GROUP,
325 CHESTNUT ST, 8TH FL, PHILADELPHIA, PA 19106 USA.
ISSN: 1024-2422.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 25

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Three novel **lipase**-producing microorganisms have been
isolated from 526 actinomycete strains by employing screening techniques
on solid media, Time-course and scale-up of enzyme production were
analyzed. The **lipases**, produced by microorganisms belonging to
the Streptomyces genus, were tested in several reactions in organic medium
using unnatural substrates. The lyophilized crude **lipases** are
stable at least for 1 month at 4 degreesC (100% recovered activity). The
lipase activity per milliliter of cell culture broth was higher
than described in the literature for other **lipases** from
actinomycetes. The three selected **lipases** displayed better
activity than commercial **lipase** from *Candida rugosa* in
the resolution of chiral secondary alcohols. The **lipase** from *S.*
halstedii also displayed very good activity in the synthesis of
carbamates.

L22 ANSWER 5 OF 59 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2001686241 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11732718
TITLE: Production of native and recombinant **lipases** by
Candida rugosa: a review.
AUTHOR: Ferrer P; Montesinos J L; Valero F; Sola C
CORPORATE SOURCE: Departament d'Enginyeria Quimica, ETSE, Universitat
Autonoma de Barcelona, Bellaterra, Spain.
SOURCE: Applied biochemistry and biotechnology, (2001 Sep) 95 (3)
221-55. Ref: 152
Journal code: 8208561. ISSN: 0273-2289.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200206
ENTRY DATE: Entered STN: 20011205
Last Updated on STN: 20020620
Entered Medline: 20020619

AB The yeast *Candida rugosa* produces multiple **lipase**
isoenzymes sharing high sequence homology but with some differences in
their catalytic properties. The regulation of *C. rugosa*
lipase (CRL) synthesis and secretion in *C. rugosa* obeys
a complex pattern. Fermentation processes for both wild-type and mutant
C. rugosa strains are available for **lipase** production.
Native CRL preparations have been extensively used for biotransformations.
However, their inherent mixture of isoforms with variable profiles
complicates interpretation and brings into question the reproducibility
achieved between preparations. Although heterologous CRLs **gene**
expression had been hampered owing to a nonuniversal codon usage, recent
advances have made heterologous CRLs available. This will expand and
improve the industrial utility of CRLs even further. The purpose of this

review is to provide a summary of the recent advances on the production of native and recombinant lipases by *C. rugosa*.

L22 ANSWER 6 OF 59 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2001269449 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11368188
TITLE: Recombinant expression and characterization of the *Candida rugosa* lip4 lipase in *Pichia pastoris*: comparison of glycosylation, activity, and stability.
AUTHOR: Tang S J; Shaw J F; Sun K H; Sun G H; Chang T Y; Lin C K; Lo Y C; Lee G C
CORPORATE SOURCE: Institute of Marine Biotechnology, National Taiwan Ocean University, Keelung, Republic of China..
SOURCE: Archives of biochemistry and biophysics, (2001 Mar 1) 387 (1) 93-8.
Journal code: 0372430. ISSN: 0003-9861.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200106
ENTRY DATE: Entered STN: 20010618
Last Updated on STN: 20010618
Entered Medline: 20010614

AB Although *Candida rugosa* utilizes a nonuniversal serine codon (CUG) for leucine, it is possible to express lipase genes (LIP) in heterologous systems. After replacing the 19 CUG codons in LIP4 with serine codons by site-directed mutagenesis, a recombinant LIP4 was functionally overexpressed in *Pichia pastoris* in this study. This recombinant glycosylated lipase was secreted into the culture medium with a high purity of 100 mg/liter in a culture broth. Purified recombinant LIP4 had a molecular mass of 60 kDa, showing a range similar to that of lipase in a commercial preparation. Since LIP4 has only a glycosylation site at position Asn-351, this position may also be the major glycosylation site in *C. rugosa* lipases. Although the thermal stability of recombinant LIP4 significantly increased from 52 to 58 degrees C after glycosylation, there were no significant differences in the catalytic properties of recombinant glycosylated lipase from *P. pastoris* and the unglycosylated one from *Escherichia coli*. These two recombinant LIP4s showed higher esterase activities toward long-chain ester (C16 and C18) and exhibited higher lipase activities toward unsaturated and long-chain lipids. In addition, LIP4 does not show interfacial activation as compared with LIP1 toward lipid substrates of tributyrin and triolein. These observations demonstrated that LIP4 shows distinguished catalytic activities with LIP1 in spite of their high sequence homology.

L22 ANSWER 7 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation. on STN
ACCESSION NUMBER: 2001:579628 SCISEARCH
THE GENUINE ARTICLE: 453ZJ
TITLE: Influence of the conformational flexibility on the kinetics and dimerisation process of two *Candida rugosa* lipase isoenzymes
AUTHOR: Pernas M A; Lopez C; Rua M L (Reprint); Hermoso J
CORPORATE SOURCE: Univ Vigo, Fac Ciencias Ourense, Area Bioquim & Biol Mol, As Lagoas S-N, Ourense 32004, Spain (Reprint); Univ Vigo, Fac Ciencias Ourense, Area Bioquim & Biol Mol, Ourense 32004, Spain; CSIC, Inst Rocasolano, Grp Cristalog Macromol & Biol Estructural, Madrid 28006, Spain
COUNTRY OF AUTHOR: Spain
SOURCE: FEBS LETTERS, (13 JUL 2001) Vol. 501, No. 1, pp. 87-91.
Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
ISSN: 0014-5793.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 32
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We have investigated the interfacial activation process of two

isoenzymes from *Candida rugosa* (Lip1 and Lip3) using triacetin as substrate. Kinetics were coupled to inhibition experiments in order to analyse the transition between the open and closed conformers. This process was slow, particularly for Lip1, in the absence of an interface provided by the substrate or a detergent. Dimers of Lip3 were also purified and their catalytic action was closer to that of a typical esterase. In spite of the high sequence homology between Lip1 and Lip3, small changes enhance hydrophobicity in the binding pocket of Lip3 and increase the flexibility of its flap. We postulated that these factors account for the higher tendency of Lip3 to dimerise fixing its open conformation. (C) 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

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ACCESSION NUMBER: 2001:575995 SCISEARCH
 THE GENUINE ARTICLE: 452RW
 TITLE: Controlling lipase enantioselectivity for organic synthesis
 AUTHOR: Berglund P (Reprint)
 CORPORATE SOURCE: Royal Inst Technol, KTH, Dept Biotechnol, SE-10044 Stockholm, Sweden (Reprint)
 COUNTRY OF AUTHOR: Sweden
 SOURCE: BIOMOLECULAR ENGINEERING, (AUG 2001) Vol. 18, No. 1, pp. 13-22.
 Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.
 ISSN: 1389-0344.
 DOCUMENT TYPE: General Review; Journal
 LANGUAGE: English
 REFERENCE COUNT: 136

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Lipases are used frequently as chiral catalysts in the synthesis of various fine chemicals and intermediates. The increasing need of compounds with high stereochemical purity requires catalysts with an improved and controlled performance. This overview emphasizes some important aspects for the control of lipase enantioselectivity and some examples where the enantioselectivity has been altered or reversed are highlighted. However, in several of these cases the complete explanation for the altered or reversed enantioselectivity remains unclear and needs to be solved. Three different strategies (engineering of the reaction medium, the substrate molecule, and the enzyme) for exploring lipase enantioselectivity at a molecular level are discussed and summarized. These three different approaches represent powerful tools for understanding the molecular basis for lipase enantioselective catalysis and can guide the rational improvement and tailoring of catalyst performance. By combining approaches from chemistry and biology much is learnt about the most important parameters controlling lipase enantioselectivity for organic synthesis. (C) 2001 Elsevier Science B.V. All rights reserved.

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ACCESSION NUMBER: 2001:264088 SCISEARCH
 THE GENUINE ARTICLE: 413CA
 TITLE: Effect of methanol and water contents on production of biodiesel fuel from plant oil catalyzed by various lipases in a solvent-free system
 AUTHOR: Kaieda M; Samukawa T; Kondo A; Fukuda H (Reprint)
 CORPORATE SOURCE: Kobe Univ, Fac Engrn, Dept Sci & Chem Engrn, Nada Ku, 1-1 Rokkodai Cho, Kobe, Hyogo 6578501, Japan (Reprint); Kobe Univ, Fac Engrn, Dept Sci & Chem Engrn, Nada Ku, Kobe, Hyogo 6578501, Japan; Kobe Univ, Grad Sch Sci & Technol, Div Mol Sci, Nada Ku, Kobe, Hyogo 6578501, Japan
 COUNTRY OF AUTHOR: Japan
 SOURCE: JOURNAL OF BIOSCIENCE AND BIOENGINEERING, (JAN 2001) Vol. 91, No. 1, pp. 12-15.
 Publisher: SOC BIOSCIENCE BIOENGINEERING JAPAN, OSAKA UNIV, FACULTY ENGINEERING, 2-1 YAMADAOKA, SUITA, OSAKA, 565-0871, JAPAN.
 ISSN: 1389-1723.

DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 7

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Methyl esters synthesized from plant oil and methanol by the methanolysis reaction are potentially important as a biodiesel fuel. The methanolysis of soybean oil by lipases from various microorganisms was investigated. Several of the lipases were found to catalyze methanolysis in a water-containing system without an organic solvent. The lipases from *Candida rugosa*, *Pseudomonas cepacia*, and *Pseudomonas fluorescens* displayed particularly high catalytic ability. The reaction rates of methanolysis catalyzed by the *C. rugosa* and *P. fluorescens* lipases decreased significantly when the water content was low, showing that water prevents the inactivation of these lipases by methanol. On the other hand, the methanolysis reaction rate catalyzed by the *P. cepacia* lipase remained high even under a low water content. In addition, the *P. cepacia* lipase gave high methyl ester contents in the reaction mixture up to 2 or 3 molar equivalents of methanol to oil, which is attributed to the *P. cepacia* lipase having substantial methanol resistance. For the same methanol content, the reaction rates of methanolysis catalyzed by the *P. cepacia* lipase increased with decreasing water content, and hence lipases strongly resistant to high methanol, such as that from *P. cepacia*, are desirable for use in methanolysis reaction processes.

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ACCESSION NUMBER: 2000:348037 SCISEARCH
THE GENUINE ARTICLE: 309JE
TITLE: Characterization of an extracellular lipase encoded by LIP2 in *Yarrowia lipolytica*
AUTHOR: Pignede G; Wang H J; Fudalej F; Gaillardin C; Seman M; Nicaud J M (Reprint)
CORPORATE SOURCE: INRA, LAB MICROBIOL & GENET MOL, GRIGNON CNRS, BP 01, F-78850 THIVERVAL GRIGNON, FRANCE (Reprint); INRA, LAB MICROBIOL & GENET MOL, GRIGNON CNRS, F-78850 THIVERVAL GRIGNON, FRANCE; LAB MAYOLY SPINDLER, SERV RECH, F-78401 CHATOU, FRANCE
COUNTRY OF AUTHOR: FRANCE
SOURCE: JOURNAL OF BACTERIOLOGY, (MAY 2000) Vol. 182, No. 10, pp. 2802-2810.
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904.
ISSN: 0021-9193.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 52

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We isolated the LIP2 gene from the lipolytic yeast *Yarrowia lipolytica*. It was found to encode a 334-amino-acid precursor protein. The secreted lipase is a 301-amino-acid glycosylated polypeptide which is a member of the triacylglycerol hydrolase family (EC 3.1.1.3). The Lip2p precursor protein is processed by the KEX2-like endoprotease encoded by XPR6. Deletion of the XPR6 gene resulted in the secretion of an active but less stable proenzyme. Thus, the pro region does not inhibit lipase secretion and activity. However, it does play an essential role in the production of a stable enzyme. Processing was found to be correct in LIP2(A) (multiple LIP2 copy integrant)-overexpressing strains, which secreted 100 times more activity than the wild type, demonstrating that XPR6 maturation was not limiting. No extracellular lipase activity was detected with the lip2 knockout (KO) strain, strongly suggesting that extracellular lipase activity results from expression of the LIP2 gene. Nevertheless, the lip2 KO strain is still able to grow on triglycerides, suggesting an alternative pathway for triglyceride utilization in *Y. lipolytica*.

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ACCESSION NUMBER: 2000:414858 SCISEARCH
 THE GENUINE ARTICLE: 317UM
 TITLE: Mutants provide evidence of the importance of glycosydic chains in the activation of **lipase 1** from *Candida rugosa*
 AUTHOR: Brocca S; Persson M; Wehtje E; Adlercreutz P; Alberghina L; Lotti M (Reprint)
 CORPORATE SOURCE: UNIV MILANO BICOCCA, DIPARTIMENTO BIOTECNOL & BIOSCI, PIAZZA SCI 2, I-20126 MILAN, ITALY (Reprint); UNIV MILANO BICOCCA, DIPARTIMENTO BIOTECNOL & BIOSCI, I-20126 MILAN, ITALY; LUND UNIV, DEPT BIOTECHNOL, S-22100 LUND, SWEDEN
 COUNTRY OF AUTHOR: ITALY; SWEDEN
 SOURCE: PROTEIN SCIENCE, (MAY 2000) Vol. 9, No. 5, pp. 985-990. Publisher: CAMBRIDGE UNIV PRESS, 40 WEST 20TH STREET, NEW YORK, NY 10011-4211. ISSN: 0961-8368.
 DOCUMENT TYPE: Article; Journal
 FILE SEGMENT: LIFE
 LANGUAGE: English
 REFERENCE COUNT: 28

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Sequence analysis of *Candida rugosa* **lipase 1** (LIP1) predicts the presence of three N-linked glycosylation sites at asparagine 291, 314, 351. To investigate the relevance of sugar chains in the activation and stabilization of LIP1, we directed site mutagenesis to replace the above mentioned asparagine with glutamine residues. Comparison of the activity of mutants with that of the wild-type (wt) **lipase** indicates that both 314 and 351 Asn to Gln substitutions influence, although at a different extent, the enzyme activity both in hydrolysis and esterification reactions, but they do not alter the enzyme water activity profiles in organic solvents or temperature stability. Introduction of Gln to replace Asn351 is likely to disrupt a stabilizing interaction between the sugar chain and residues of the inner side of the lid in the enzyme active conformation. The effect of deglycosylation at position 314 is more difficult to explain and might suggest a more general role of the sugar moiety for the structural stability of **lipase 1**. Conversely, Asn291Gln substitution does not affect the lipolytic or the esterase activity of the mutant that behaves essentially as the wt enzyme. This observation supports the hypothesis that changes in activity of Asn314Gln and Asn351Gln mutants are specifically due to deglycosylation.

L22 ANSWER 12 OF 59 MEDLINE on STN DUPLICATE 3
 ACCESSION NUMBER: 2001073206 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 11049754
 TITLE: Recombinant expression of the *Candida rugosa* **lip4** **lipase** in *Escherichia coli*.
 AUTHOR: Tang S J; Sun K H; Sun G H; Chang T Y; Lee G C
 CORPORATE SOURCE: Institute of Marine Biotechnology, Keelung, 20224, Taiwan.. tsj@mail.ntou.edu.tw
 SOURCE: Protein expression and purification, (2000 Nov) 20 (2) 308-13. Journal code: 9101496. ISSN: 1046-5928.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 200101
 ENTRY DATE: Entered STN: 20010322
 Last Updated on STN: 20010322
 Entered Medline: 20010103

AB It is difficult to express recombinant *Candida rugosa* **lipases** (CRLs) in heterologous systems, since *C. rugosa* utilizes a nonuniversal serine codon CUG for leucine. In this study, recombinant LIP4 in which all 19 CUG codons had been converted to a universal serine codon was overexpressed in *Escherichia coli* BL21(DE3). The recombinant LIP4 was found mainly in the inclusion bodies and showed a low catalytic activity. To increase the amount of soluble form and activity of recombinant LIP4, the DNA was fused to the gene for thioredoxin (TrxFus-LIP4) and then expressed in *E. coli* strain AD494(DE3). This strategy promotes the formation of disulfide bonds in the cytosol and yields enzymatically active forms of LIP4. The

purified recombinant TrxFus-LIP4 and LIP4 expressed in AD494(DE3) had the same catalytic profiles. In addition, recombinant LIP4 had higher esterase activities toward long-chain ester and lower **lipase** activities toward tributyrin, triolein, and olive oil. This system for the expression of fungal **lipase** in *E. coli* strain AD494(DE3) is reliable and may produce enzymatically active forms of recombinant **lipase** without an in vitro refolding procedure.
Copyright 2000 Academic Press.

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ACCESSION NUMBER: 2001:107432 BIOSIS
DOCUMENT NUMBER: PREV200100107432
TITLE: Purification and characterization of Lip2 and Lip3 isoenzymes from a *Candida rugosa* pilot-plant scale fed-batch fermentation.
AUTHOR(S): Pernas, M. A.; Lopez, C.; Pastrana, L.; Rua, M. L. [Reprint author]
CORPORATE SOURCE: Area de Bioquímica e Biología Molecular, Facultade de Ciencias de Ourense, Universidade de Vigo, As Lagoas, 32004, Ourense, Spain
mlrua@uvigo.es
SOURCE: Journal of Biotechnology, (30 December, 2000) Vol. 84, No. 2, pp. 163-174. print.
CODEN: JBITD4. ISSN: 0168-1656.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 28 Feb 2001
Last Updated on STN: 15 Feb 2002

AB Previous purification of a crude extracellular enzyme preparation from *Candida rugosa* ATCC 14830 pilot-plant fed-batch fermentations showed the presence of two **lipase** isoenzymes, Lip2 and Lip3, differing in their molecular masses (58 and 62 kDa, respectively). These enzymes were purified but the **lipases** were forming active aggregates with a molecular mass higher than 200 kDa. In this work we developed a purification method following three steps: ammonium sulfate precipitation, sodium cholate treatment and ethanol/ether precipitation, and anion exchange chromatography which allowed the sequential disaggregation of the isoenzymes. Pure and monomeric Lip2 and Lip3 were characterized according to pI, glycosylation and activity for p-nitrophenol esters and triacylglycerols of varying acyl chain. Lip3 was the best catalyst for the hydrolysis of the simple esters and triacylglycerols with short and medium acyl chains.

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ACCESSION NUMBER: 2001:49257 BIOSIS
DOCUMENT NUMBER: PREV200100049257
TITLE: Design and realization of a tailor-made enzyme to modify the molecular recognition of 2-arylpropionic esters by *Candida rugosa* **lipase**.
AUTHOR(S): Manetti, Fabrizio; Mileto, Daniela; Corelli, Federico; Soro, Simonetta; Palocci, Cleofe; Cernia, Enrico; D'Acquarica, Ilaria; Lotti, Marina; Alberghina, Lilia; Botta, Maurizio [Reprint author]
CORPORATE SOURCE: Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, Via Aldo Moro snc, I-53100, Siena, Italy
botta@unisi.it
SOURCE: Biochimica et Biophysica Acta, (30 November, 2000) Vol. 1543, No. 1, pp. 146-158. print.
CODEN: BBACAQ. ISSN: 0006-3002.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Jan 2001
Last Updated on STN: 12 Feb 2002

AB Within a research project aimed at probing the substrate specificity and the enantioselectivity of *Candida rugosa* **lipase** (CRL), computer modeling studies of the interactions between CRL and methyl (+)-2-(3-benzoylphenyl)propionate (Ketoprofen methyl ester) have been carried out in order to identify which amino acids are essential to the enzyme/substrate interaction. Different binding models of the substrate

enantiomers to the active site of CRL were investigated by applying a computational protocol based on molecular docking, conformational analysis, and energy minimization procedures. The structural models of the computer generated complexes between CRL and the substrates enabled us to propose that Phe344 and Phe345, in addition to the residues constituting the catalytic triad and the oxyanion hole, are the amino acids mainly involved in the enzyme-ligand interactions. To test the importance of these residues for the enzymatic activity, site-directed mutagenesis of the selected amino acids has been performed, and the mutated enzymes have been evaluated for their conversion and selectivity capabilities toward different substrates. The experimental results obtained in these biotransformation reactions indicate that Phe344 and especially Phe345 influence CRL activity, supporting the findings of our theoretical simulations.

L22 ANSWER 15 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:207906 CAPLUS
DOCUMENT NUMBER: 132:346651
TITLE: Reversible enzyme immobilization via a very strong and nondistorting ionic adsorption on support-polyethylenimine composites
AUTHOR(S): Mateo, Cesar; Abian, Olga; Fernandez-Lafuente, Roberto; Guisan, Jose M.
CORPORATE SOURCE: Departamento de Biocatálisis, Instituto de Catálisis, CSIC, Madrid, 28049, Spain
SOURCE: Biotechnology and Bioengineering (2000), 68(1), 98-105
CODEN: BIBIAU; ISSN: 0006-3592
PUBLISHER: John Wiley & Sons, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB New tailor-made anionic exchange resins have been prep'd., based on films of large polyethylenimine polymers (e.g., mol. wt. 25,000) completely coating, via covalent immobilization, the surface of different porous supports (agarose, silica, polymeric resins). Most proteins contained in crude exts. from different sources have been very strongly adsorbed on them. Ionic exchange properties of such composites strongly depend on the size of polyethylenimine polymers as well as on the exact conditions of the covalent coating of the solids with the polymer. On the contrary, similar coating protocols yield similar matrixes by using different porous supports as starting material. For example, 77% of all proteins contained in crude exts. from *Escherichia coli* were adsorbed, at low ionic strength, on the best matrixes, and less than 15% of the adsorbed proteins were eluted from the support in the presence of 0.3 M NaCl. Under these conditions, 100% of the adsorbed proteins were eluted from conventional DEAE supports. Such polyethylenimine-support composites were also very suitable to perform very strong and nondistorting reversible immobilization of industrial enzymes. For example, lipase from *Candida rugosa* (CRL), β -galactosidase from *Aspergillus oryzae* and D-amino acid oxidase (DAAO) from *Rhodotorula gracilis*, were adsorbed on such matrixes in a few minutes at pH 7.0 and 4.degree.C. Immobilized enzymes preserved 100% of catalytic activity and remained fully immobilized in 0.2 M NaCl. In addn. to that, CRL and DAAO were highly stabilized upon immobilization. Stabilization of DAAO, a dimeric enzyme, seems to be due to the involvement of both enzyme sub-units in the ionic adsorption.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 16 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:222764 CAPLUS
DOCUMENT NUMBER: 133:55154
TITLE: *Candida rugosa* lipases: from molecular evolution analysis to the design of a synthetic gene
AUTHOR(S): Lotti, Marina; Alberghina, Lilia
CORPORATE SOURCE: Dipartimento Biotechnologie e Bioscienze, Università Degli Studi Milano-Bicocca, Milan, 20126, Italy
SOURCE: Protein Engineering in Industrial Biotechnology (2000), 63-74. Editor(s): Alberghina, Lilia. Harwood Academic Publishers: Amsterdam, Neth.
CODEN: 68TUAF

DOCUMENT TYPE: Conference; General Review
LANGUAGE: English

AB A review with 60 refs. The family of lipase enzymes (CRLs) synthesized by the fungus *Candida rugosa* provides issues of interest for protein engineers because of a few unique features. First of all, *C. rugosa* lipases are members of a large set of proteins closely related in both sequence and function. In an evolutionary perspective, one can consider CRK proteins as the result of a successful process of mutagenesis where a defined group of amino acid changes have been pos. selected since they gave rise to functional variants. The study of lipase sequences therefore may provide a good starting point towards the design of functional mutants. On the other hand, *C. rugosa* belongs to a subgroup of *Candida* species which, using a non-conventional decoding of one serine codon, requires the development of novel approaches for the heterologous expression of its genes. Information arising may therefore be of interest for researchers using non-conventional expression systems.

REFERENCE COUNT: 60 THERE ARE 60 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 17 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1999:220064 CAPLUS

DOCUMENT NUMBER: 130:247858

TITLE: A synthetic LIPI gene of *Candida rugosa* with the codon usage of *Pichia pastoris* and the manufacture of the lipase encoded by the gene

INVENTOR(S): Brocca, Stefania; Schmidt-Dannert, Claudia; Lotti, Marina; Alberghina, Lilia; Schmid, Rolf

PATENT ASSIGNEE(S): Unilever N.V., Neth.

SOURCE: PCT Int. Appl., 44 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------|--|----------|-----------------|----------|
| WO 9914338 | A1 | 19990325 | WO 1997-NL524 | 19970916 |
| W: | AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | |
| RW: | GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG | | | |
| AU 9742249 | A1 | 19990405 | AU 1997-42249 | 19970916 |
| EP 1012301 | A1 | 20000628 | EP 1997-940483 | 19970916 |
| R: | BE, DE, DK, FR, GB, NL, SE | | | |

PRIORITY APPLN. INFO.: WO 1997-NL524 A 19970916

AB The dimorphic yeast *Candida rugosa* has an unusual codon-usage which hampers the functional expression of genes derived from this yeast in a conventional heterologous host. Lipases produced by this yeast are extensively used in industrial bioconversions, but com. lipase samples contain several different isoforms encoded by the LIP genes family. In a first laborious attempt the LIPI gene, encoding the major isoform of the *C. rugosa* lipases (CRLs), was systematically modified by site-directed mutagenesis to gain functional expression in *S. cerevisiae*. Mutagenesis was designed by homol. with other lipases. As alternative approach, the gene (1688 bp) was completely synthesized with an optimized nucleotide sequence in terms of heterologous expression in yeast and simplified genetic manipulation. The synthetic gene was overexpressed in *Pichia pastoris*. The lipase was produced at high level and purity, accounting for 90-95 % of the secreted proteins. The phys.-chem. and catalytic properties of the recombinant lipase were compared with those of a com. *C. rugosa* lipase prepn.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 18 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.
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ACCESSION NUMBER: 1999:938866 SCISEARCH
THE GENUINE ARTICLE: 260KL
TITLE: Switched enantioference of Humicola lipase
for 2-phenoxyalkanoic acid ester homologs can be
rationalized by different substrate binding modes
AUTHOR: Berglund P (Reprint); Vallikivi I; Fransson L; Dannacher
H; Holmquist M; Martinelle M; Bjorkling F; Parve O; Hult K
CORPORATE SOURCE: ROYAL INST TECHNOL, DEPT BIOTECHNOL, SE-10044 STOCKHOLM,
SWEDEN (Reprint); TALLINN TECH UNIV, INST CHEM, DEPT
BIOORGAN CHEM, TALLINN 12618, ESTONIA; LEO PHARMACEUT
PROD, DK-2750 BALLERUP, DENMARK
COUNTRY OF AUTHOR: SWEDEN; ESTONIA; DENMARK
SOURCE: TETRAHEDRON-ASYMMETRY, (29 OCT 1999) Vol. 10, No. 21, pp.
4191-4202.
Publisher: PERGAMON-ELSEVIER SCIENCE LTD, THE BOULEVARD,
LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND.
ISSN: 0957-4166.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: PHYS
LANGUAGE: English
REFERENCE COUNT: 32

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Humicola lanuginosa lipase was used for enantioselective
hydrolyses of a series of homologous 2-phenoxyalkanoic acid ethyl esters.
The enantioselectivity (E-value) of the enzyme changed from an
(R)-enantiomer preference for the smallest substrate, 2-phenoxypropanoic
acid ester, to an (S)-enantiomer preference for the homologous esters with
longer acyl moieties. The E-values span the range from E=13 (R) to E=56
(S). A molecular modeling study identified two different substrate-binding
modes for each enantiomer. We found that the enantiomers favored different
modes. This discovery provided a model that offered a rational explanation
for the observed switch in enantioselectivity. (C) 1999 Elsevier Science
Ltd. All rights reserved.

L22 ANSWER 19 OF 59 MEDLINE on STN DUPLICATE 4

ACCESSION NUMBER: 1999402725 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10473391
TITLE: Analysis of the gene family encoding
lipases in Candida rugosa by competitive
reverse transcription-PCR.
AUTHOR: Lee G C; Tang S J; Sun K H; Shaw J F
CORPORATE SOURCE: Institute of Biochemistry, National Yang-Ming University,
Taipei, Taiwan 11211.
SOURCE: Applied and environmental microbiology, (1999 Sep) 65 (9)
3888-95.
Journal code: 7605801. ISSN: 0099-2240.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF025307
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991223

AB Synthesis of multiple extracellular lipases in Candida
rugosa has been demonstrated. However, it is difficult to
characterize the expression spectrum of lip genes, since the
sequences of the lip multigene family are very closely related. A
competitive reverse transcription-PCR assay was developed to quantify the
expression of lip genes. In agreement with the protein profile,
the abundance of lip mRNAs was found to be (in decreasing order) lip1,
lip3, lip2, lip5, and lip4. To analyze the effects of different culture
conditions, the transcript concentrations for these mRNA species were
normalized relative to the values for gpd, encoding glyceraldehyde-3-
phosphate dehydrogenase. In relative terms, lip1 and lip3 were highly and
constitutively expressed (about 10(5) molecules per microg of total RNA)

whereas the other inducible lip genes, especially lip4, showed significant changes in mRNA expression under different culture conditions. These results indicate that differential transcriptional control of lip genes results in multiple forms of lipase proteins.

L22 ANSWER 20 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.
on STN

ACCESSION NUMBER: 1999:812285 SCISEARCH

THE GENUINE ARTICLE: 247EW

TITLE: Bacterial biocatalysts: Molecular biology, three-dimensional structures, and biotechnological applications of lipases

AUTHOR: Jaeger K E (Reprint); Dijkstra B W; Reetz M T

CORPORATE SOURCE: RUHR UNIV BOCHUM, LEHRSTUHL BIOL MIKROORGANISMEN, D-44780 BOCHUM, GERMANY (Reprint); UNIV GRONINGEN, BIOPHYS CHEM LAB, NL-9747 AG GRONINGEN, NETHERLANDS; MAX PLANCK INST KOHLENFORSCH, D-45470 MULHEIM, GERMANY

COUNTRY OF AUTHOR: GERMANY; NETHERLANDS

SOURCE: ANNUAL REVIEW OF MICROBIOLOGY, (JUL-AUG 1999) Vol. 53, pp. 315-&.

Publisher: ANNUAL REVIEWS INC, 4139 EL CAMINO WAY, PO BOX 10139, PALO ALTO, CA 94303-0139.

ISSN: 0066-4227.

DOCUMENT TYPE: General Review; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 169

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Bacteria produce and secrete lipases, which can catalyze both the hydrolysis and the synthesis of long-chain acylglycerols. These reactions usually proceed with high regioselectivity and enantioselectivity, and, therefore, Lipases have become very important stereoselective biocatalysts used in organic chemistry. High-level production of these biocatalysts requires the understanding of the mechanisms underlying gene expression, folding, and secretion. Transcription of Lipase genes may be regulated by quorum sensing and two-component systems; secretion can proceed either via the Sec-dependent general secretory pathway or via ABC transporters. In addition, some lipases need folding catalysts such as the lipase-specific foldases and disulfide-bond-forming proteins to achieve a secretion-competent conformation. Three-dimensional structures of bacterial lipases were solved to understand the catalytic mechanism of lipase reactions. Structural characteristics include an alpha/beta hydrolase fold, a catalytic triad consisting of a nucleophilic serine located in a highly conserved Gly-X-Ser-X-Gly pentapeptide, and an aspartate or glutamate residue that is hydrogen bonded to a histidine. Four substrate binding pockets were identified for triglycerides: an oxyanion hole and three pockets accommodating the fatty acids bound at positions sn-1, sn-2, and sn-3. The differences in size and the hydrophilicity/hydrophobicity of these pockets determine the enantiopreference of a lipase. The understanding of structure-function relationships will enable researchers to tailor new lipases for biotechnological applications. At the same time, directed evolution in combination with appropriate screening systems will be used extensively as a novel approach to develop lipases with high stability and enantioselectivity.

L22 ANSWER 21 OF 59 MEDLINE on STN DUPLICATE 5

ACCESSION NUMBER: 2000039909 MEDLINE

DOCUMENT NUMBER: PubMed ID: 10570248

TITLE: Structure and conformational flexibility of Candida rugosa lipase.

AUTHOR: Cygler M; Schrag J D

CORPORATE SOURCE: Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount Avenue, Montreal, Quebec, Canada.. mirek.cygler@bri.nrc.ca

SOURCE: Biochimica et biophysica acta, (1999 Nov 23) 1441 (2-3) 205-14. Ref: 23

Journal code: 0217513. ISSN: 0006-3002.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199912
ENTRY DATE: Entered STN: 20000114
Last Updated on STN: 20000114
Entered Medline: 19991230

AB Three-dimensional structures of a number of lipases determined in the past decade have provided a solid structural foundation for our understanding of lipase function. The structural studies of *Candida rugosa* lipase summarized here have addressed many facets of interfacial catalysis. These studies have revealed a fold and catalytic site common to other lipases. Different conformations likely to correlate with interfacial activation of the enzyme were observed in different crystal forms. The structures of enzyme-inhibitor complexes have identified the binding site for the scissile fatty acyl chain, provided the basis for molecular modeling of triglyceride binding and provided insight into the structural basis of the common enantiopreferences shown by lipases.

L22 ANSWER 22 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.
on STN

ACCESSION NUMBER: 1999:413237 SCISEARCH

THE GENUINE ARTICLE: 198XT

TITLE: A controlled fed-batch cultivation for the production of new crude lipases from *Candida rugosa* with improved properties in fine chemistry

AUTHOR: Sanchez A; Ferrer P; Serrano A; Valero F (Reprint); Sola C; Pernas M; Rua M L; FernandezLafuente R; Guisan J M; delaCasa R; Sinisterra J V; SanchezMontero J M

CORPORATE SOURCE: UNIV AUTONOMA BARCELONA, DETP ENGN QUIM, E-08193 BARCELONA, SPAIN (Reprint); UNIV AUTONOMA BARCELONA, DETP ENGN QUIM, E-08193 BARCELONA, SPAIN; UNIV VIGO, FAC CIENCIAS OURENSE, AREA BIOQUIM & BIOLOXIA MOL, ORENSE 32004, SPAIN; UNIV COMPLUTENSE, FAC FARM, DEPT QUIM ORGAN & FARMACEUT, E-28040 MADRID, SPAIN; CSIC, INST CATALISIS, LAB TECNOL ENZIMAT, E-28049 MADRID, SPAIN

COUNTRY OF AUTHOR: SPAIN

SOURCE: JOURNAL OF BIOTECHNOLOGY, (15 APR 1999) Vol. 69, No. 2-3, pp. 169-182.

Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS.

ISSN: 0168-1656.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE; AGRI

LANGUAGE: English

REFERENCE COUNT: 43

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A controlled constant feeding rate fed-batch strategy using oleic acid as inducer produced a crude lipase preparation from *Candida rugosa* (CRL-UAB) with higher protein content, carbohydrate content and lipase activity than commercial Sigma type VII CRL. CRL-UAB was partially characterised and tested in selective biotransformations of chiral compounds in aqueous (2-hydroxy 4-phenyl butanoic acid ethyl ester (HPBE)) and organic media (2-phenyl propionic acid and ketoprofen). CRL-UAB showed higher substrate specificity and enantioselectivity in aqueous media compared to Sigma CRL. Also, higher specific initial rates with 2-phenyl propionic acid and ketoprofen were observed in organic media. The influence of water on the esterification of ketoprofen was not relevant with CRL-UAB under the conditions tested, whereas a dramatic influence was observed in Sigma CRL. Different CRL-UAB batches obtained under the same cultivation controlled conditions were identical from the point of view of chromatographic behaviour, immobilisation rates and catalytic properties, indicating that a reproducible *C. rugosa* lipase extract had been obtained. (C) 1999 Elsevier Science: B.V. All rights reserved.

L22 ANSWER 23 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.
on STN

ACCESSION NUMBER: 1998:915281 SCISEARCH

THE GENUINE ARTICLE: 142NR
 TITLE: Structure and activity of rat pancreatic lipase
 -related protein 2
 AUTHOR: Roussel A; Yang Y Q; Ferrato F; Verger R; Cambillau C;
 Lowe M (Reprint)
 CORPORATE SOURCE: WASHINGTON UNIV, SCH MED, DEPT PEDIAT, ST LOUIS, MO 63110
 (Reprint); WASHINGTON UNIV, SCH MED, DEPT PEDIAT, ST
 LOUIS, MO 63110; WASHINGTON UNIV, SCH MED, DEPT MOL BIOL &
 PHARMACOL, ST LOUIS, MO 63110; CNRS, IFR1, UPR 9039,
 F-13402 MARSEILLE 20, FRANCE; CNRS, IFR1, UPR 9025, LAB
 LIPOLYSE ENZYMAT, F-13402 MARSEILLE 20, FRANCE
 COUNTRY OF AUTHOR: USA; FRANCE
 SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (27 NOV 1998) Vol. 273,
 No. 48, pp. 32121-32128.
 Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC,
 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.
 ISSN: 0021-9258.
 DOCUMENT TYPE: Article; Journal
 FILE SEGMENT: LIFE
 LANGUAGE: English
 REFERENCE COUNT: 39

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The pancreas expresses several members of the lipase
 gene family including pancreatic triglyceride lipase
 (PTL) and two homologous proteins, pancreatic lipase-related
 proteins 1 and 2 (PLRP1 and PLRP2). Despite their similar amino acid
 sequences, PTL, PLRP1, and PLRP2 differ in important kinetic properties.
 PLRP1 has no known activity. PTL and PLRP2 differ in substrate
 specificity, bile acid inhibition, colipase requirement, and interfacial
 activation. To begin understanding the structural explanations for these
 functional differences, we solved the crystal structure of rat (r)PLRP2
 and further characterized its kinetic properties. The 1.8 Angstrom
 structure of rPLRP2, like the tertiary structure of human PTL, has a
 globular N-terminal domain and a beta-sandwich C-terminal domain. The lid
 domain occupied the closed position, suggesting that rPLRP2 should show
 interfacial activation. When we reexamined this issue with tripropionin as
 substrate, rPLRP2 exhibited interfacial activation. Because the active
 site topology of rPLRP2 resembled that of human PTL, we predicted and
 demonstrated that the lipase inhibitors E600 and
 tetrahydrolipstatin inhibit rPLRP2. Although PTL and rPLRP2 have similar
 active sites, rPLRP2 has a broader substrate specificity that we confirmed
 using a monolayer technique. With this assay, we showed for the first time
 that rPLRP2 prefers phosphatidylglycerol and ethanolamine over
 phosphatidylcholine. In summary, we confirmed and extended the observation
 that PLRP2 lipases have a broader substrate specificity than
 PTL, we demonstrated that PLRP2 lipases show interfacial
 activation, and we solved the first crystal structure of a PLRP2
 lipase that contains a lid domain.

L22 ANSWER 24 OF 59 MEDLINE on STN DUPLICATE 6
 ACCESSION NUMBER: 1998318052 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 9655346
 TITLE: Design, total synthesis, and functional overexpression of
 the *Candida rugosa* lip1 gene coding for
 a major industrial lipase.
 AUTHOR: Brocca S; Schmidt-Dannert C; Lotti M; Alberghina L; Schmid
 R D
 CORPORATE SOURCE: Institut fur Technische Biochemie, Universitat Stuttgart,
 Germany.
 SOURCE: Protein science : a publication of the Protein Society,
 (1998 Jun) 7 (6) 1415-22.
 Journal code: 9211750. ISSN: 0961-8368.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199809
 ENTRY DATE: Entered STN: 19980910
 Last Updated on STN: 19980910
 Entered Medline: 19980901

AB The dimorphic yeast *Candida rugosa* has an unusual codon usage

that hampers the functional expression of **genes** derived from this yeast in a conventional heterologous host. Commercial samples of *C. rugosa* lipase (CRL) are widely used in industry, but contain several different isoforms encoded by the lip gene family, among which the isoform encoded by the **gene** lip1 is the most prominent. In a first laborious attempt, the lip1 **gene** was systematically modified by site-directed mutagenesis to gain functional expression in *Saccharomyces cerevisiae*. As alternative approach, the **gene** (1647 bp) was completely synthesized with an optimized nucleotide sequence in terms of heterologous expression in yeast and simplified genetic manipulation. The synthetic **gene** was functionally expressed in both hosts *S. cerevisiae* and *Pichia pastoris*, and the effect of heterologous leader sequences on expression and secretion was investigated. In particular, using *P. pastoris* cells, the synthetic **gene** was functionally overexpressed, allowing for the first time to produce recombinant Lip1 of high purity at a level of 150 U/mL culture medium. The physicochemical and catalytic properties of the recombinant lipase were compared with those of a commercial, nonrecombinant *C. rugosa* lipase preparation containing lipase isoforms.

L22 ANSWER 25 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.
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ACCESSION NUMBER: 1998:132513 SCISEARCH

THE GENUINE ARTICLE: YV746

TITLE: A cold-adapted lipase of an Alaskan psychrotroph, *Pseudomonas* sp. strain B11-1: **Gene** cloning and enzyme purification and characterization

AUTHOR: Choo D W; Kurihara T; Suzuki T; Soda K; Esaki N (Reprint)

CORPORATE SOURCE: KYOTO UNIV, INST CHEM RES, KYOTO 611, JAPAN (Reprint); KYOTO UNIV, INST CHEM RES, KYOTO 611, JAPAN; KANSAI UNIV, FAC ENGN, OSAKA 564, JAPAN

COUNTRY OF AUTHOR: JAPAN

SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (FEB 1998) Vol. 64, No. 2, pp. 486-491.
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.
ISSN: 0099-2240.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE; AGRI

LANGUAGE: English

REFERENCE COUNT: 44

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A psychrotrophic bacterium producing a cold-adapted lipase upon growth th at low temperatures was isolated from Alaskan soil and identified as a *Pseudomonas* strain, The lipase **gene** (lipP) was cloned from the strain and sequenced, The amino acid sequence deduced from the nucleotide sequence of the **gene** (924 bp) corresponded to a protein of 308 amino acid residues with a molecular weight of 33,714. LipP also has consensus motifs conserved in other cold-adapted lipases, i.e., Lipase 2 from Antarctic *Moraxella* TA144 (G. Feller, M. Thiry, J. L. Arpigny, and C. Gerday, *DNA Cell Biol.* 10:381-388, 1991) and the mammalian hormone-sensitive lipase (D. Langin, H. Laurell, L. S. Hoist, P. Belfrage, and C. Holm, *Proc. Natl. Acad. Sci. USA* 90:4897-4901, 1993): a pentapeptide, GDSAG, containing the putative active-site serine and an BG dipeptide, LipP was purified from an extract of recombinant *Escherichia coli* C600 cells harboring a plasmid coding for the lipP **gene**. The enzyme showed a 1,3-positional specificity toward triolein, p-Nitrophenyl esters of fatty acids with short to medium chains (C-4 and C-6) served as good substrates. The enzyme was stable between pH 6 and 9, and the optimal pH for the enzymatic hydrolysis of tributyrin was around 8, The activation energies for the hydrolysis of p-nitrophenyl butyrate and p-nitrophenyl laurate were determined to be 11.2 and 7.7 kcal/mol, respectively. in the temperature range 5 to 35 degrees C, The enzyme was unstable at temperatures higher than 45 degrees C. The K-m of the enzyme for p-nitrophenyl butyrate increased with increases in the assay temperature, The enzyme was strongly inhibited by Zn²⁺, Cu²⁺, Fe³⁺, and Hg²⁺ but was not affected by phenylmethylsulfonyl fluoride and bis-nitrophenyl phosphate, Various water-miscible organic solvents, such as methanol and dimethyl sulfoxide, at concentrations of 0 to 30%

(vol/vol) activated the enzyme.

L22 ANSWER 26 OF 59 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 1998386725 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9720256
TITLE: Physiological control on the expression and secretion of
Candida rugosa lipase.
AUTHOR: Lotti M; Monticelli S; Montesinos J L; Brocca S; Valero F;
Lafuente J
CORPORATE SOURCE: Dipartimento di Fisiologia e Biochimica Generali,
Universita degli Studi di Milano, Italy..
lotti@imiucca.csi.unimi.it
SOURCE: Chemistry and physics of lipids, (1998 Jun) 93 (1-2) 143-8.
Journal code: 0067206. ISSN: 0009-3084.
PUB. COUNTRY: Ireland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19981006
Last Updated on STN: 19981006
Entered Medline: 19980918

AB The fungus Candida rugosa secretes an extracellular
lipase whose production is induced by the addition of fatty acids
to the culture broth. This lipase is indeed composed by several
protein isoforms partly differing in their catalytic properties.
Synthesis and secretion of lipase proteins by C. rugosa
cells were studied in culture media containing either glucose or oleic
acid as the carbon source. It was shown that, according to their
regulation, lipase-encoding genes might be grouped in
two classes, one of which is constitutively expressed and the other is
induced by fatty acids. The synthesis of inducible enzymes is inhibited
at the level of transcription by the addition of glucose and, conversely,
oleic acid appears to hinder the synthesis of the constitutive
lipase. Growth conditions supporting high level expression both
in batch and in continuous culture give rise to the intracellular
accumulation of enzyme, possibly due to the existence of a rate-limiting
step in the transport of the newly synthesized protein. These results
suggest the possibility to develop fermentation processes aimed at the
control of the enzyme composition.

L22 ANSWER 27 OF 59 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 1998386718 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9720249
TITLE: Characterization of the Candida rugosa
lipase system and overexpression of the lip1
isoenzyme in a non-conventional yeast.
AUTHOR: Mileto D; Brocca S; Lotti M; Takagi M; Alquati C;
Alberghina L
CORPORATE SOURCE: Department of General Physiology and Biochemistry,
University of Milano, Italy.
SOURCE: Chemistry and physics of lipids, (1998 Jun) 93 (1-2) 47-55.
Ref: 40
Journal code: 0067206. ISSN: 0009-3084.
PUB. COUNTRY: Ireland
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199809
ENTRY DATE: Entered STN: 19981006
Last Updated on STN: 19981006
Entered Medline: 19980918

AB The fungus C. rugosa produces lipase isoenzymes (CRLs)
homologous to the Geotrichum candidum and Yarrowia lipolytica
lipases to which they share ca. 40 and 30% sequence identity, with
a domain of sequence conservation at the N-terminal half of the protein.
CRL proteins have high sequence homology but are not identical in their
catalytic activity, therefore calling for the resolution of isoforms via
heterologous expression. The non-conventional use of a serine codon in

several *Candida* species frustrates overexpression in the currently available host systems. The **LIP1 gene**, coding for the major CRL form, was therefore expressed in *C. maltosa*, a related fungus with the same codon usage as *C. rugosa*. A recombinant **lipase** was produced and secreted in an active form in the culture medium upon engineering the 5' and 3' ends of the **gene**.

L22 ANSWER 28 OF 59 MEDLINE on STN DUPLICATE 9
 ACCESSION NUMBER: 1999127041 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 9928080
 TITLE: A toolbox of recombinant **lipases** for industrial applications.
 AUTHOR: Schmidt-Dannert C; Pleiss J; Schmid R D
 CORPORATE SOURCE: Institute of Technical Biochemistry, University of Stuttgart, Germany.
 SOURCE: Annals of the New York Academy of Sciences, (1998 Dec 13) 864 14-22. Ref: 27
 Journal code: 7506858. ISSN: 0077-8923.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 General Review; (REVIEW)
 (REVIEW, TUTORIAL)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199903
 ENTRY DATE: Entered STN: 19990324
 Last Updated on STN: 19990324
 Entered Medline: 19990309

AB We created a toolbox of recombinant, microbial **lipases**, which allows us in combination with a **lipase** database to choose among the overexpressed **lipases** the most appropriate for a specific application and to improve it further via mutagenesis. By systematic comparison of geometry and properties of the scissile fatty acid binding site of five representative **lipases** of each family of structurally homologous **lipases**, three subgroups can be defined. Hence, efficient expression systems for the functional production of large amounts of microbial **lipases**, representing different **lipase** subgroups, were developed. In particular, recombinant **lipases** from *Bacillus thermocatenulatus* and *Pseudomonas cepacia* were functionally overexpressed in *E. coli*. The **lipase genes** from *Geotrichum candidum* CMICC 335426 and *Rhizopus oryzae* were overexpressed in *Pichia pastoris*. Due to an unusual codon usage that prevents heterologous expression, the **LIP1 gene** (1647 nt) of *Candida rugosa* was completely synthesized and overexpressed in *Pichia pastoris*.

L22 ANSWER 29 OF 59 MEDLINE on STN DUPLICATE 10
 ACCESSION NUMBER: 1998112187 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 9450388
 TITLE: Molecular cloning of **cDNA** for the zeta isoform of the 14-3-3 protein: homologous sequences in the 3'-untranslated region of frog and human zeta isoforms.
 AUTHOR: Miura I; Nakajima T; Ohtani H; Kashiwagi A; Nakamura M
 CORPORATE SOURCE: Laboratory for Amphibian Biology, Faculty of Science, Hiroshima University, Japan.
 SOURCE: Zoological science, (1997 Oct) 14 (5) 771-5.
 Journal code: 8702287. ISSN: 0289-0003.
 PUB. COUNTRY: Japan
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Biotechnology
 ENTRY MONTH: 199802
 ENTRY DATE: Entered STN: 19980306
 Last Updated on STN: 19980306
 Entered Medline: 19980224

AB 14-3-3 proteins constitute a family of well-conserved eukaryotic proteins that possess diverse biochemical activities such as regulation of **gene** transcription, cell proliferation and activation of protein kinase C. At least 7 subtypes (alpha to theta) of 14-3-3 protein are known, but the zeta subtype of this protein has been cloned only in mammals. We cloned the zeta subtype of 14-3-3 protein (14-3-3 zeta) from

the frog, *Rana rugosa*. The sequence encoded 245 amino acids that share 92% identity with rat and bovine 14-3-3 zeta s, and 92% with human phospholipase A2 (PLA2; 14-3-3 zeta). Northern blot analysis revealed a single band of about 1.8 kb in tadpoles at stage 25. The 14-3-3 zeta mRNA level was high in the brain, lung, spleen and kidney, and low in the heart and testis, as opposed to the mRNA level, which was only faintly detected in the liver, pancreas, ovary and muscle. Furthermore, high similarity in the 3'-untranslated region (3'-UTR) was observed between frog and human 14-3-3 zeta cDNA. The results suggest that 14-3-3 zeta is highly conserved throughout eukaryotic evolution, and that the homologous sequence in the 3'-UTR of 14-3-3 zeta cDNA may be conserved in frogs and humans.

L22 ANSWER 30 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.
on STN

ACCESSION NUMBER: 97:769505 SCISEARCH
THE GENUINE ARTICLE: BJ65Z
TITLE: Kinetics, molecular modeling, and synthetic applications with microbial lipases
AUTHOR: Hult K (Reprint); Holmquist M
CORPORATE SOURCE: ROYAL INST TECHNOL, DEPT BIOCHEM & BIOTECHNOL, S-10044 STOCKHOLM, SWEDEN (Reprint)
COUNTRY OF AUTHOR: SWEDEN
SOURCE: METHODS IN ENZYMOLOGY, (30 SEP 1997) Vol. 286, pp. 386-405

Publisher: ACADEMIC PRESS INC, 525 B STREET, SUITE 1900, SAN DIEGO, CA 92101-4495.
ISSN: 0076-6879.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 47

L22 ANSWER 31 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.
on STN

ACCESSION NUMBER: 97:728198 SCISEARCH
THE GENUINE ARTICLE: BJ57Q
TITLE: Stereoselectivity of lipase from *Rhizopus oryzae* toward triacylglycerols and analogs: Computer-aided modeling and experimental validation
AUTHOR: Haalck L (Reprint); Paltauf F; Pleiss J; Schmid R D; Spener F; Stadler P
CORPORATE SOURCE: INST CHEM & BIOCHEM SENSOR RES, D-48149 MUNSTER, GERMANY (Reprint); GRAZ TECH UNIV, DEPT BIOCHEM & FOOD CHEM, A-8010 GRAZ, AUSTRIA; UNIV STUTTGART, INST TECH BIOCHEM, D-70569 STUTTGART, GERMANY
COUNTRY OF AUTHOR: GERMANY; AUSTRIA
SOURCE: METHODS IN ENZYMOLOGY, (15 SEP 1997) Vol. 284, pp. 353-376

Publisher: ACADEMIC PRESS INC, 525 B STREET, SUITE 1900, SAN DIEGO, CA 92101-4495.
ISSN: 0076-6879.
DOCUMENT TYPE: General Review; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 40

L22 ANSWER 32 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 11

ACCESSION NUMBER: 1997:712688 CAPLUS
DOCUMENT NUMBER: 128:11432
TITLE: Cloning, sequencing, and expression of *Candida rugosa* lipases
AUTHOR(S): Alberghina, Lilia; Lotti, Marina
CORPORATE SOURCE: USA
SOURCE: Methods in Enzymology (1997), 284(Lipases, Part A), 246-260
CODEN: MENZAU; ISSN: 0076-6879
PUBLISHER: Academic
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The authors describe work concerning the cloning and expression of *Candida*

rugosa lipases, which are peculiar among fungal lipase for two reasons: (1) the large no. of encoding genes and (2) a deviation from the universal genetic code in the source yeast. The importance and effects of this phenomenon for lipase structure and function, as well as for the expression of cloned genes in host systems, are discussed.

REFERENCE COUNT: 25 THERE ARE 25 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 33 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.
on STN

ACCESSION NUMBER: 97:186489 SCISEARCH

THE GENUINE ARTICLE: WK598

TITLE: The crystal structure of a triacylglycerol lipase from *Pseudomonas cepacia* reveals a highly open conformation in the absence of a bound inhibitor

AUTHOR: Kim K K; Song H K; Shin D H; Hwang K Y; Suh S W (Reprint)

CORPORATE SOURCE: SEOUL NATL UNIV, COLL NAT SCI, DEPT CHEM, SEOUL 151742, SOUTH KOREA (Reprint); SEOUL NATL UNIV, COLL NAT SCI, DEPT CHEM, SEOUL 151742, SOUTH KOREA

COUNTRY OF AUTHOR: SOUTH KOREA

SOURCE: STRUCTURE, (15 FEB 1997) Vol. 5, No. 2, pp. 173-185.

Publisher: CURRENT BIOLOGY LTD, 34-42 CLEVELAND STREET, LONDON, ENGLAND W1P 6LB.

ISSN: 0969-2126.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 61

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: Lipases, a family of enzymes which catalyze the hydrolysis of triglycerides, are widely distributed in many organisms. True lipases are distinguished from esterases by the characteristic interfacial activation they exhibit at an oil-water interface. Lipases are one of the most frequently used biocatalysts for organic reactions performed under mild conditions. Their biotechnological applications include food and oil processing and the preparation of chiral intermediates for the synthesis of enantiomerically pure pharmaceuticals. Recent structural studies on several lipases have provided some clues towards understanding the mechanisms of hydrolytic activity, interfacial activation, and stereoselectivity. This study was undertaken in order to provide structural information on bacterial lipases, which is relatively limited in comparison to that on the enzymes from other sources.

Results: We have determined the crystal structure of a triacylglycerol lipase from *Pseudomonas cepacia* (Pet) in the absence of a bound inhibitor using X-ray crystallography. The structure shows the lipase to contain an alpha/beta-hydrolase fold and a catalytic triad comprising of residues Ser87, His286 and Asp264. The enzyme shares several structural features with homologous lipases from *Pseudomonas glumae* (PgL) and *Chromobacterium viscosum* (CvL), including a calcium-binding site. The present structure of Pet reveals a highly open conformation with a solvent-accessible active site. This is in contrast to the structures of PgL and Pet in which the active site is buried under a closed or partially opened 'lid', respectively.

Conclusions: Pet exhibits some structural features found in other lipases. The presence of the Ser-His-Asp catalytic triad, an oxyanion hole, and the opening of a helical lid suggest that this enzyme shares the same mechanisms of catalysis and interfacial activation as other lipases. The highly open conformation observed in this study is likely to reflect the activated form of the lipase at an oil-water interface. The structure suggests that the interfacial activation of bacterial lipases involves the reorganization of secondary structures and a large movement of the lid to expose the active site. This is similar to the mechanism described for other well characterized fungal and mammalian lipases.

L22 ANSWER 34 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 12

ACCESSION NUMBER: 1997:343890 CAPLUS

DOCUMENT NUMBER: 127:61390

TITLE: The evolution of a non universal codon as detected in

AUTHOR(S): Candida rugosa lipase
 Alberghina, Lilia; Lotti, Marina
 CORPORATE SOURCE: Department of General Physiology and Biochemistry,
 University of Milano, Milan, I-20133, Italy
 SOURCE: Journal of Molecular Catalysis B: Enzymatic (1997),
 3(1-4), 37-41
 CODEN: JMCEF8; ISSN: 1381-1177
 PUBLISHER: Elsevier
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB In several Candida species belonging to the same monophyletic group, CUG -
 one of the six triplets coding leucine in the universal genetic code - is
 read as an addnl. codon for serine. CUG for serine is rarely employed but
 in the lipase genes of Candida rugosa, where
 it is the major serine codon. This yeast secretes multiple lipase
 isoenzymes (CRLs) tightly related in their amino acid sequence. CRL
 proteins contain 16 to 19 CUG-serines comprehensive of Ser 209 in the
 catalytic center and other serines having an obvious structural role. In
 this paper, results obtained from sequence anal. and mutagenesis are
 discussed and shown to be consistent with an evolutionary pathway in which
 the codon was reassigned via ambiguous decoding by a novel seryl-tRNA
 whose abundance conferred pos. selection pressure for the extensive use of
 CUG as a serine codon.
 REFERENCE COUNT: 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS
 RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L22 ANSWER 35 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.
 on STN

ACCESSION NUMBER: 97:740032 SCISEARCH
 THE GENUINE ARTICLE: XY802
 TITLE: High-level production of recombinant Geotrichum candidum
 lipases in yeast Pichia pastoris
 AUTHOR: Holmquist M (Reprint); Tessier D C; Cygler M
 CORPORATE SOURCE: NATL RES COUNCIL CANADA, BIOTECHNOL RES INST, 6100
 ROYALMOUNT AVE, MONTREAL, PQ H4P 2R2, CANADA (Reprint);
 ROYAL INST TECHNOL, DEPT BIOCHEM & BIOTECHNOL, S-10044
 STOCKHOLM, SWEDEN
 COUNTRY OF AUTHOR: CANADA; SWEDEN
 SOURCE: PROTEIN EXPRESSION AND PURIFICATION, (OCT 1997) Vol. 11,
 No. 1, pp. 35-40.
 Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525
 B ST, STE 1900, SAN DIEGO, CA 92101-4495.
 ISSN: 1046-5928.
 DOCUMENT TYPE: Article; Journal
 FILE SEGMENT: LIFE
 LANGUAGE: English
 REFERENCE COUNT: 30

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We describe the heterologous high-level expression of the two
 Geotrichum candidum lipase (GCL) isoenzymes from strain ATCC
 34614 in the methylotrophic yeast Pichia pastoris. The Lipase
 cDNAs were placed under the control of the methanol-inducible
 alcohol oxidase promoter. The lipases expressed in P. pastoris
 were fused to the a-factor secretion signal peptide of Saccharomyces
 cerevisiae and were secreted into the culture medium. Cultures of P.
 pastoris expressing lipase accumulated active recombinant enzyme
 in the supernatant to levels of similar to 60 mg/L virtually free from
 contaminating proteins. This yield exceeds that previously reported with
 S. cerevisiae by a factor of more than 60. Recombinant GCL I and GCL II
 had molecular masses of similar to 63 and similar to 66 kDa, respectively,
 as determined by SDS-PAGE. The result of endoglucosidase H digestion
 followed by Western blot analysis of the lipases suggested that
 the enzymes expressed in P. pastoris received N-linked high-mannose-type
 glycosylation to an extent, 6-8% (w/w), similar to that in G. candidum.
 The specific activities and substrate specificities of both recombinant
 lipases were determined and were found to agree with what has been
 reported for the enzymes isolated from the native source. (C) 1997
 Academic Press.

L22 ANSWER 36 OF 59 MEDLINE on STN DUPLICATE 13
 ACCESSION NUMBER: 1998051607 MEDLINE

DOCUMENT NUMBER: PubMed ID: 9390168
 TITLE: Isolation of carboxylester lipase (CEL)
 isoenzymes from *Candida rugosa* and identification
 of the corresponding genes.
 AUTHOR: Diczfalussy M A; Hellman U; Alexson S E
 CORPORATE SOURCE: Department of Medical Laboratory Sciences and Technology,
 Karolinska Institutet, Huddinge University Hospital,
 Sweden.
 SOURCE: Archives of biochemistry and biophysics, (1997 Dec 1) 348
 (1) 1-8.
 Journal code: 0372430. ISSN: 0003-9861.
 PUB. COUNTRY: United States
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199712
 ENTRY DATE: Entered STN: 19980116
 Last Updated on STN: 19980116
 Entered Medline: 19971224

AB The yeast *Candida rugosa* produces extracellular lipases
 which are widely used for industrial purposes. A commercial
 lipase preparation from this yeast can be separated into several
 isoenzymes which differ in carbohydrate content, isoelectric point,
 substrate specificity, and primary sequence. We have here purified and
 characterized three lipases, which also hydrolyze p-nitrophenyl
 esters, from a commercial preparation of this yeast. These three
 carboxylester lipases (CELs) elute differently on hydrophobic
 interaction chromatography, and have different carbohydrate contents and
 substrate specificities. Sequence analysis of their amino termini and
 peptides generated by LysC treatment showed that CEL-1 and CEL-3 probably
 have identical primary structure while CEL-2 was proven to be a different
 enzyme. Sequence comparison showed that both CEL-1 and CEL-3 are products
 of the LIP1 gene and that CEL-2 is the gene product of
 LIP2, cloned by Longhi et al. (Biochim. Biophys. Acta 1131, 227-232,
 1992).

L22 ANSWER 37 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1996:304153 CAPLUS
 DOCUMENT NUMBER: 124:334869
 TITLE: Transgenic oil plants expressing a foreign
 lipase gene and their use in the
 manufacture of free fatty acids
 INVENTOR(S): Alibert, Gilbert; Mouloungui, Zephirin; Boudet, Alain
 PATENT ASSIGNEE(S): Institut National Polytechnique de Toulouse
 (I.N.P.T.), Fr.
 SOURCE: PCT Int. Appl., 31 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: French
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|--|------|----------|-----------------|------------|
| WO 9603511 | A2 | 19960208 | WO 1995-FR957 | 19950718 |
| WO 9603511 | A3 | 19960425 | | |
| W: AU, BG, CA, CN, HU, JP, NZ, PL, RO, RU, US | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| FR 2722798 | A1 | 19960126 | FR 1994-9272 | 19940725 |
| FR 2722798 | B1 | 19960913 | | |
| CA 2195560 | AA | 19960208 | CA 1995-2195560 | 19950718 |
| AU 9529849 | A1 | 19960222 | AU 1995-29849 | 19950718 |
| EP 770134 | A2 | 19970502 | EP 1995-925897 | 19950718 |
| R: AT, DE, ES, FR, GB, IT | | | | |
| US 5942659 | A | 19990824 | US 1997-776210 | 19970124 |
| PRIORITY APPLN. INFO.: | | | FR 1994-9272 | A 19940725 |
| | | | WO 1995-FR957 | W 19950718 |

AB A method of producing fatty acids or their derivs. from oil plants without
 the need for hydrolysis of oils with added exogenous lipase is
 described. Transgenic plants with a lipase gene under
 the control of a tissue-specific or inducible promoter are constructed.

The tissue-specific promoter drives expression outside the oil-accumulating tissues, the inducible promoter may be one that can be induced shortly before processing of the plant material. Upon processing the material, the breakdown of the plant tissue leads to mixing of the lipase and the lipids with a resulting hydrolysis and release of fatty acids or their derivatives from the lipids. A gene for a microbial lipase, e.g. from *Candida*, *Rhizopus*, or *Pseudomonas*, that is widely used in industrial fat hydrolysis may be used. The construction of a transgenic *Brassica napus* expressing a *Rhizopus niveus* lipase cDNA from a napin promoter is described.

L22 ANSWER 38 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.
on STN

ACCESSION NUMBER: 96:886619 SCISEARCH

THE GENUINE ARTICLE: VV002

TITLE: Facile purification of highly active recombinant

Staphylococcus hyicus lipase fragment and

characterization of a putative lid region

AUTHOR: Chang R C; Chen J C; Shaw J F (Reprint)

CORPORATE SOURCE: ACAD SINICA, INST BOT, NANKANG 11529, TAIPEI, TAIWAN

(Reprint); ACAD SINICA, INST BOT, NANKANG 11529, TAIPEI,

TAIWAN; CHINA JR COLL MARINE TECHNOL, DEPT SEA FOOD

TECHNOL, TAIPEI 111, TAIWAN; NATL TAIWAN OCEAN UNIV, INST

MARINE BIOTECHNOL, CHILUNG 20224, TAIWAN

COUNTRY OF AUTHOR: TAIWAN

SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (21
NOV 1996) Vol. 228, No. 3, pp. 774-779.

Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525
B ST, STE 1900, SAN DIEGO, CA 92101-4495.

ISSN: 0006-291X.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 28

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A fragment of *Staphylococcus hyicus* lipase gene

(Ala248 to Ala640) was inserted into plasmid pET20(b+). The resulting His-tagged recombinant protein (49 kDa) was overexpressed in *Escherichia coli* BL21(DE3) as an highly active lipase and was purified by nickel-coupled resin. Site-directed mutagenesis showed that in comparison with wild type enzyme, the L326F and L326A enzymes showed a 3.4 and 5 fold increase in the K_m , respectively, but only a 44% and a 64% decrease in the k_{cat}/K_m , respectively, suggesting that Leu326 of the putative lid participated in substrate-binding. (C) 1996 Academic Press, Inc.

L22 ANSWER 39 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 14

ACCESSION NUMBER: 1996:212601 CAPLUS

DOCUMENT NUMBER: 124:287140

TITLE: Effect of the leader sequence on the expression of
recombinant *C. rugosa* lipase by *S.*

cerevisiae cells

AUTHOR(S): Fusetti, Fabrizia; Brocca, Stefania; Porro, Danilo;
Lotti, Marina

CORPORATE SOURCE: Dipartimento Fisiologia Biochimica Generali,
Universita Studi Milano, Milan, 20133, Italy

SOURCE: Biotechnology Letters (1996), 18(3), 281-6

CODEN: BILED3; ISSN: 0141-5492

PUBLISHER: Chapman and Hall

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The *Candida rugosa* lipase I gene was

expressed in *Saccharomyces cerevisiae*. The recombinant lipase was efficiently synthesized only following the replacement of the enzyme endogenous leader sequence with the signal peptide of the *Kluyveromyces fragilis* killer toxin. Amt. of accumulated lipase was 10-20 mg/L in batch culture and >1 g/L in a computer-controlled fed-batch fermenter system.

L22 ANSWER 40 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1996:333944 CAPLUS

DOCUMENT NUMBER: 125:28683

TITLE: Protein engineering of a fungal **lipase**:
towards expression of a recombinant *Candida*
rugosa lipase

AUTHOR(S): Alberghina, Lilia; Lotti, Marina

CORPORATE SOURCE: Dipartimento Fisiologia e Biochimica Generali,
Universita' degli Studi di Milano, Milan, 20133, Italy

SOURCE: NATO ASI Series, Series E: Applied Sciences (1996),
317(Engineering of/with Lipases), 219-228
CODEN: NAESDI; ISSN: 0168-132X

PUBLISHER: Kluwer

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review with 20 refs.

L22 ANSWER 41 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.
on STN

ACCESSION NUMBER: 96:221118 SCISEARCH

THE GENUINE ARTICLE: TN944

TITLE: A STRUCTURAL BASIS FOR ENANTIOSELECTIVE INHIBITION OF
CANDIDA-RUGOSA LIPASE BY LONG-CHAIN
ALIPHATIC-ALCOHOLS

AUTHOR: HOLMQUIST M; HAEFFNER F; NORIN T; HULT K (Reprint)

CORPORATE SOURCE: ROYAL INST TECHNOL, DEPT BIOCHEM & BIOTECHNOL,
TEKNIKRINGEN 34, S-10044 STOCKHOLM, SWEDEN (Reprint);
ROYAL INST TECHNOL, DEPT CHEM, S-10044 STOCKHOLM, SWEDEN

COUNTRY OF AUTHOR: SWEDEN

SOURCE: PROTEIN SCIENCE, (JAN 1996) Vol. 5, No. 1, pp. 83-88.
ISSN: 0961-8368.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: ENGLISH

REFERENCE COUNT: 31

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Molecular modeling showed that the enantiomers of heptyl
2-methyldecanoate are productively bound to the active site of *Candida*
rugosa lipase in quite different conformations. The
fast-reacting S-enantiomer may well occupy the previously identified
acyl-binding tunnel in the active site of the **lipase**. By
contrast, the slow-reacting R-enantiomer must be bound to the active site,
leaving the tunnel empty to allow the formation of two catalytically
essential hydrogen bonds between His 449 of the catalytic triad and the
transition state of the catalyzed reaction. This information enables us to
propose a molecular mechanism explaining how long-chain aliphatic alcohols
act as enantioselective inhibitors of this **lipase** in the
resolution of 2-methyldecanoic acid. Long-chain aliphatic alcohols may
coordinate to the acyl-binding tunnel of the *C. rugosa*
lipase, thereby selectively inhibiting the turnover of the
fast-reacting S-enantiomer, thus resulting in a lowered enantioselectivity
in the resolution.

L22 ANSWER 42 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.
on STN

ACCESSION NUMBER: 97:5816 SCISEARCH

THE GENUINE ARTICLE: VX982

TITLE: Studying the active site pocket of *Staphylococcus hyicus*
lipase by site-directed mutagenesis

AUTHOR: Chang R C; Chen J C; Shaw J F (Reprint)

CORPORATE SOURCE: NATL TAIWAN OCEAN UNIV, INST MARINE BIOTECHNOL, CHILUNG
20224, TAIWAN (Reprint); NATL TAIWAN OCEAN UNIV, INST
MARINE BIOTECHNOL, CHILUNG 20224, TAIWAN; CHINA JR COLL
MARINE TECHNOL, DEPT SEA FOOD TECHNOL, TAIPEI 111, TAIWAN;
ACAD SINICA, INST BOT, TAIPEI 11529, TAIWAN

COUNTRY OF AUTHOR: TAIWAN

SOURCE: BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (4
DEC 1996) Vol. 229, No. 1, pp. 6-10.
Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS, 525
B ST, STE 1900, SAN DIEGO, CA 92101-4495.
ISSN: 0006-291X.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 33

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Site-directed mutagenesis of a previously constructed, recombinant *Staphylococcus hyicus* lipase (49 kDa) showed that Val363 played a role in catalysis and substrate-binding. In comparison with wild type enzyme, the 64% and 89% decrease in the catalytic efficiency (kcat/K-m) of the V363N and V363A enzymes, respectively, were largely caused by a 3.5- and 5.5-fold increase in the substrate-binding affinity (K-m), respectively. In comparison with wild type enzyme, a G371A enzyme showed a 40% decrease in the k(m), suggesting that G371 was important for substrate-binding specificity. Site-directed mutagenesis of the active site Asp559 revealed that in comparison with wild type enzyme, a D559E enzyme exhibited a 47% decrease in the kcat/K-m, but a twofold increase in the K-m for p-nitrophenyl butyrate, suggesting that Asp-559, a component of the catalytic triad, was involved in substrate-specificity. (C) 1996 Academic Press, Inc.

L22 ANSWER 43 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1995:645247 CAPLUS
DOCUMENT NUMBER: 123:29030
TITLE: Lipase-labelled probe
INVENTOR(S): Pittner, Fritz; Schalkhammer, Thomas; Ecker, Bernhard; Kynclova, Eva; Wakolbinger, Werner
PATENT ASSIGNEE(S): Boehringer Mannheim GmbH, Germany
SOURCE: PCT Int. Appl., 30 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: German
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|--|------|----------------|-----------------|----------|
| WO 9510775 | A1 | 19950420 | WO 1994-EP3379 | 19941013 |
| W: AU, CA, JP, NZ, US | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| CA 2151731 | AA | 19950420 | CA 1994-2151731 | 19941013 |
| AU 9478557 | A1 | 19950504 | AU 1994-78557 | 19941013 |
| AU 671392 | B2 | 19960822 | | |
| JP 07509618 | T2 | 19951026 | JP 1994-511295 | 19941013 |
| EP 679257 | A1 | 19951102 | EP 1994-929543 | 19941013 |
| R: AT, CH, DE, ES, FR, GB, IT, LI | | | | |
| PRIORITY APPLN. INFO.: | | AT 1993-2071 | | 19931015 |
| | | WO 1994-EP3379 | | 19941013 |

AB To improve the thermal and chem. stability of an enzyme-labeled probe, a lipase that is preferably extd. from *Candida rugosa* and whose isoenzymes or structural analogs have at least 70% amino acid homol. and lipase activity is used as the enzyme.

L22 ANSWER 44 OF 59 MEDLINE on STN

DUPLICATE 15

ACCESSION NUMBER: 96155626 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8575019
TITLE: Localization of lipase genes on *Candida rugosa* chromosomes.
AUTHOR: Brocca S; Grandori R; Breviario D; Lotti M
CORPORATE SOURCE: Dipartimento di Fisiologia e Biochimica Generali, Italia.
SOURCE: Current genetics, (1995 Oct) 28 (5) 454-7.
Journal code: 8004904. ISSN: 0172-8083.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199603
ENTRY DATE: Entered STN: 19960321
Last Updated on STN: 19960321
Entered Medline: 19960314

AB In the yeast *Candida rugosa* the lipase isozymes are encoded by a family of genes, five of which have been cloned and sequenced in our laboratory. In this paper we report on the identification and preliminary characterization of two new related sequences, thus extending this multigene family to seven members. The

total DNA content of *Candida* cells was estimated by laser flow-cytometry at about 20 Mb. Eight chromosomes with sizes ranging between 100 kb and 2.1 Mb, as determined by comparison with *S. cerevisiae* chromosomal bands, were resolved by pulsed-field gel electrophoresis. The lipase-encoding genes were localized on chromosome I, therefore suggesting that they have originated through multiple duplication events of an ancestral gene.

L22 ANSWER 45 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1995:502091 CAPLUS
DOCUMENT NUMBER: 123:75827
TITLE: Oligonucleotide labeled lipase as a new sensitive hybridization probe and its use in bio-assays and biosensors
AUTHOR(S): Kynclova, Eva; Hartig, Andreas; Schalkhammer, Thomas
CORPORATE SOURCE: Inst. Biochem. Mol. Zellbiol., Univ. Vienna, Vienna, A-1030, Austria
SOURCE: Journal of Molecular Recognition (1995), 8(1/2), 139-45
CODEN: JMORE4; ISSN: 0952-3499
PUBLISHER: Wiley
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Radiolabeled polynucleotide probes have been employed extensively for the detection of complementary nucleic acids by specific hybridization. Within the last few years, various methods have been developed using enzyme-labeled probes to avoid unstable and hazardous isotopes. These assays, based on photometry, fluorescence, and chemiluminescence, have helped to overcome the use of radioactive probes. To increase the performance of a non-radioactive DNA detection system, the labeling enzyme should remain stable under hybridization conditions which allow the formation of a 15-25 bp long DNA-DNA duplex ($T_m = 50-70^\circ\text{C}$). Therefore, the use of unstable phosphatase and peroxidase conjugates must be avoided due to the compn. of the hybridization mixt. and the high temp. By screening various hydrolytic enzymes to fit the special demands, fungal lipases turned out to be the most practical. They offer high sensitivity due to an extremely high turnover no., stability at room temp. for several years, thermostability under working conditions and an easy design of various chromogenic, fluorescent and electrochem. active substrates. Several types of silanized, oxidized and unmodified metal sensors and also std. microtiter plates modified with amino groups were used for the immobilization of oligonucleotides. A sandwich hybridization using the lipase-labeled oligonucleotide probe and a terminal immobilized capture DNA on a microtiter plate or sensor surface combined with a rapid hybridization in soln. simplifies and improves the performance of the DNA detection system.

L22 ANSWER 46 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1995:291461 CAPLUS
DOCUMENT NUMBER: 122:283357
TITLE: Expression of recombinant *Candida rugosa* lipase
AUTHOR(S): Lotti, M.; Brocca, S.; Fusetti, F.; Alberghina, L.
CORPORATE SOURCE: Fisiologia e Biochimica Generali, Univ. di Milano, Milan, 20133, Italy
SOURCE: Mededelingen - Faculteit Landbouwkundige en Toegepaste Biologische Wetenschappen (Universiteit Gent) (1994), 59(4B), 2313-19
CODEN: MFLBER; ISSN: 1373-7503
DOCUMENT TYPE: Journal
LANGUAGE: English

AB In the asporogenic yeast *Candida rugosa* lipase is encoded by a family of genes. As a consequence, multiple lipase isoenzymes with similar but not identical mol. and catalytic properties are secreted in the culture medium. The gene LIP1, coding for the major lipase protein, was engineered by replacement of its leader sequence and expressed in *S. cerevisiae* under the control of an inducible promoter. Levels of expression as high as 20 mg/L of glycosylated intracellular protein were obtained.

L22 ANSWER 47 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.
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ACCESSION NUMBER: 94:656877 SCISEARCH
THE GENUINE ARTICLE: PK830
TITLE: COMPUTER MODELING OF SUBSTRATE-BINDING TO LIPASES
FROM RHIZOMUCOR-MIEHEI, HUMICOLA-LANUGINOSA, AND CANDIDA-
RUGOSA
AUTHOR: NORIN M; HAEFFNER F; ACHOUR A; NORIN T; HULT K (Reprint)
CORPORATE SOURCE: ROYAL INST TECHNOL, DEPT BIOCHEM & BIOTECHNOL, S-10044
STOCKHOLM, SWEDEN (Reprint); ROYAL INST TECHNOL, DEPT
BIOCHEM & BIOTECHNOL, S-10044 STOCKHOLM, SWEDEN; ROYAL
INST TECHNOL, DEPT CHEM, S-10044 STOCKHOLM, SWEDEN
COUNTRY OF AUTHOR: SWEDEN
SOURCE: PROTEIN SCIENCE, (SEP 1994) Vol. 3, No. 9, pp. 1493-1503.
ISSN: 0961-8368.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: 42

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The substrate-binding sites of the triacyl glyceride lipases
from Rhizomucor miehei, Humicola lanuginosa, and Candida rugosa
were studied by means of computer modeling methods. The space around the
active site was mapped by different probes. These calculations suggested 2
separate regions within the binding site. One region showed high affinity
for aliphatic groups, whereas the other region was hydrophilic. The
aliphatic site should be a binding cavity for fatty acid chains. Water
molecules are required for the hydrolysis of the acyl enzyme, but are
probably not readily accessible in the hydrophobic interface, in which
lipases are acting. Therefore, the hydrophilic site should be
important for the hydrolytic activity of the enzyme.

Lipases from R. miehei and H. lanuginosa are excellent
catalysts for enantioselective resolutions of many secondary alcohols. We
used molecular mechanics and dynamics calculations of enzyme-substrate
transition-state complexes, which provided information about molecular
interactions important for the enantioselectivities of these reactions.

L22 ANSWER 48 OF 59 MEDLINE on STN DUPLICATE 16
ACCESSION NUMBER: 95094819 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8001575
TITLE: Pancreatic carboxylester lipase from Atlantic
salmon (Salmo salar). cDNA sequence and
computer-assisted modelling of tertiary structure.
AUTHOR: Gjellesvik D R; Lorens J B; Male R
CORPORATE SOURCE: Laboratory for Marine Molecular Biology, University of
Bergen, Norway.
SOURCE: European journal of biochemistry / FEBS, (1994 Dec 1) 226
(2) 603-12.
Journal code: 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L23929
ENTRY MONTH: 199501
ENTRY DATE: Entered STN: 19950215
Last Updated on STN: 20000303
Entered Medline: 19950126

AB We report the isolation and characterization of a 1795-bp cDNA
fragment encoding Atlantic salmon pancreatic carboxylester lipase
from salmon pancreas mRNA. The nearly full-length cDNA
contained a 540-amino-acid open-reading frame, encompassing the mature
protein (by similarity to mammalian carboxylester lipase
enzymes). The salmon carboxylester lipase primary structure
shared 58% identity with mammalian carboxylester lipases,
lacking the proline-rich C-terminal repeats found in human and rat
carboxylester lipases. Congruent with other esterase B type
enzymes, the salmon carboxylester lipase contained a canonical
serine-esterase catalytic triad motif consisting of serine, histidine and
aspartic acid. Computer-assisted modelling of the tertiary structure for
salmon carboxylester lipase was conducted using acetylcholine

esterase (*Torpedo californica*) as a template structure. The model, in conjunction with sequence comparisons and available enzymological data, has been used to locate putative bile-salt-binding and lipid-binding sites. The carboxylester lipase enzymes contain a unique, highly conserved insert region that may be associated with bile-salt binding. In the model structure, this region is located close to the active site, and contains a tyrosine residue with an adjacent carboxylester-lipase-conserved arginine. These traits have previously been predicted for the non-specific (regarding bile-salt hydroxylation) bile-salt-binding site in carboxylester lipase enzymes. At this site, a dihydroxy or trihydroxy bile-salt molecule may bind the tyrosine via hydrophobic interactions, the anionic bile-salt head group may bind the arginine, while hydrogen bonding between the bile-salt 12 alpha hydroxy group and an adjacent asparagine residue is possible. The model does not contain an active site 'lid' structure as found in other lipases. The carboxylester lipase structural homolog to the 'flap' of the lipases from *Geotrichum candidum* and *Candida rugosa* contains a carboxylester-lipase-conserved deletion that renders this region unable to cover the active site. Instead, the shortening of this loop leads to solvent exposure of the carboxylester lipase insert region, an additional indication of the functional importance of this region.

L22 ANSWER 49 OF 59 MEDLINE on STN DUPLICATE 17
 ACCESSION NUMBER: 94302002 MEDLINE
 DOCUMENT NUMBER: PubMed ID: 8029208
 TITLE: Variability within the *Candida rugosa* lipases family.
 AUTHOR: Lotti M; Tramontano A; Longhi S; Fusetti F; Brocca S; Pizzi E; Alberghina L
 CORPORATE SOURCE: Dipartimento Fisiologia e Biochimica Generali, Universita degli Studi di Milano, Italy.
 SOURCE: Protein engineering, (1994 Apr) 7 (4) 531-5.
 Journal code: 8801484. ISSN: 0269-2139.
 PUB. COUNTRY: ENGLAND: United Kingdom
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: Priority Journals
 ENTRY MONTH: 199408
 ENTRY DATE: Entered STN: 19940818
 Last Updated on STN: 19940818
 Entered Medline: 19940808

AB Several fungi secrete lipase isozymes differing in biochemical properties and in some cases in substrate specificity. In the yeast *Candida rugosa*, a family of related genes encodes for multiple lipase proteins, highly homologous in sequence but partially different in the regions interacting with the substrate molecule. Analysis of these substitutions performed on the basis of multiple alignments and using a 3-D model of the enzyme, allows identification of a restricted number of amino acids possibly involved in substrate specificity of *Candida* lipases.

L22 ANSWER 50 OF 59 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 1993:503929 BIOSIS
 DOCUMENT NUMBER: PREV199396127936
 TITLE: Chemoenzymatic synthesis of pure enantiomeric 2-arylpropionic acids.
 AUTHOR(S): Garcia, Mariano; Del Campo, Carmen; Llama, Emilio F.; Sanchez-Montero, Jose M.; Sinisterra, Jose V. [Reprint author]
 CORPORATE SOURCE: Dep. Organic, Pharmaceutical Chemistry, Faculty Pharmacy, Univ. Complutense, 28040 Madrid, Spain
 SOURCE: Tetrahedron, (1993) Vol. 49, No. 37, pp. 8433-8440.
 CODEN: TETRAB. ISSN: 0040-4020.
 DOCUMENT TYPE: Article
 LANGUAGE: English
 ENTRY DATE: Entered STN: 5 Nov 1993
 Last Updated on STN: 6 Nov 1993

AB A new chemoenzymatic procedure to obtain pure enantiomeric 2-arylpropionic acids is described. The one pot synthesis of (+-)-2-arylpropionic acids

is carried out by addition of dichlorocarbene to the C dbd O bond of arylmethylketones and hydrogenolysis of the addition product. The racemic mixture is resolved by enantiospecific hydrolysis of the racemic ethyl esters using native lipase from *Candida rugosa*. The good yields, the accessibility of the starting arylmethylketones and the stereospecificity of the enzymatic hydrolysis make the process interesting in order to obtain the same non steroidal antiinflammatory drugs such as Ibuprofen or Naproxen.

L22 ANSWER 51 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1993:553043 CAPLUS

DOCUMENT NUMBER: 119:153043

TITLE: Structure of a family of lipase-encoding genes from *Candida cylindracea*

AUTHOR(S): Fusetti, F.; Lotti, M.; Brocca, S.; Longhi, S.; Alberghina, L.

CORPORATE SOURCE: Dip. Fisiol. Biochim. Gen., Univ. Milano, Milan, I-20133, Italy

SOURCE: Mededelingen van de Faculteit Landbouwwetenschappen, Universiteit Gent (1992), 57(4b), 2045-52
CODEN: MFLRA3; ISSN: 0368-9697

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Five genes coding for lipase have been isolated from the yeast *Candida cylindracea* and their nucleotide sequences have been detd. The deduced amino acid sequences correspond to 534 residues mature proteins, each preceded by signal sequences for secretion. Lipase proteins, although related by high sequence homol., differ in their isoelec. points and in glycosylation sites. Based on the high sequence homol. with the *Geotrichum candidum* lipases and with *Torpedo californica* acetylcholinesterase, a structural model of *C. cylindracea* lipase was inferred. The authors propose that *C. cylindracea* lipases have a Ser-Glu-His catalytic triad, with the active serine surrounded by a Y/FGESAG consensus sequence and a structural organization very similar to that of both ref. enzymes.

L22 ANSWER 52 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1993:403830 CAPLUS

DOCUMENT NUMBER: 119:3830

TITLE: Cloning and nucleotide sequences of two lipase genes from *Candida cylindracea*

AUTHOR(S): Longhi, Sonia; Fusetti, Fabrizia; Grandori, Rita; Lotti, Marina; Vanoni, Marco; Alberghina, Lilia

CORPORATE SOURCE: Dip. Fisiol., Univ. Studi Milano, Milan, Italy

SOURCE: Biochimica et Biophysica Acta (1992), 1131(2), 227-32
CODEN: BBACAQ; ISSN: 0006-3002

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Two lipase-encoding genes (LIP1 and LIP2) were isolated from a SacI genomic library of the yeast *C. cylindracea* and their nucleotide sequences were detd. Comparison with the sequence of a cDNA ruled out the presence of introns in the 2 genes. Both ORFs encode for mature proteins of 534 residues with putative signal peptides of 15 and 14 amino acids, resp. When compared with other lipase sequences, the 2 *C. cylindracea* lipases showed homol. only with the *Geotrichum candidum* lipase, whereas they shared a significant similarity with several esterases.

L22 ANSWER 53 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1993:35111 CAPLUS

DOCUMENT NUMBER: 118:35111

TITLE: Homology-derived three-dimensional structure prediction of *Candida cylindracea* lipase

AUTHOR(S): Longhi, Sonia; Lotti, Marina; Fusetti, Fabrizia; Pizzi, Elisabetta; Tramontano, Anna; Alberghina, Lilia

CORPORATE SOURCE: Dip. di Fisiol. e Biochim. Gen., Sez. di Biochim. Comp., Univ. degli Stud. di Milano, Milan, Italy

SOURCE: Biochimica et Biophysica Acta (1992), 1165(1), 129-33
CODEN: BBACAQ; ISSN: 0006-3002

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A structural model of *C. cylindracea* lipase (CCL) is proposed based on the reported x-ray structure of the highly homologous *Geotrichum candidum* lipase (GCL). The network of interactions around the active site, the salt and disulfide bridge pattern is conserved in the proposed structure. Functional, structural and evolutionary aspects of the peculiar usage of CTG codons by *C. cylindracea* ATCC 14830 are discussed.

L22 ANSWER 54 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1992:167539 CAPLUS
DOCUMENT NUMBER: 116:167539
TITLE: Molecular cloning of a lipase and of a lipase-related gene from *Candida cylindracea*
AUTHOR(S): Alberghina, Lilia; Grandori, Rita; Longhi, Sonia; Lotti, Marina; Fusetti, Fabrizia; Vanoni, Marco
CORPORATE SOURCE: Dip. Fisiol. Biochim. Gen., Univ. Milano, Milan, 20133, Italy
SOURCE: GBF Monographs (1991), 16(Lipases), 231-5
CODEN: GBMOEB; ISSN: 0930-4320
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The screening of genomic libraries from *C. cylindracea* with synthetic oligonucleotides has led to the isolation of 2 lipase-related sequences. They have been characterized by restriction mapping and partially sequenced. One of them contains a lipase-coding sequence.

L22 ANSWER 55 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1993:140913 CAPLUS
DOCUMENT NUMBER: 118:140913
TITLE: The structure of lipase genes and pseudogenes of *Candida cylindracea*
AUTHOR(S): Kawaguchi, Yoshiyuki; Honda, Hiroshi
CORPORATE SOURCE: Tokyo Res. Lab., Meito Sangyo Co. Ltd., Hachioji, 192, Japan
SOURCE: GBF Monographs (1991), 16(Lipases), 221-30
CODEN: GBMOEB; ISSN: 0930-4320
DOCUMENT TYPE: Journal
LANGUAGE: English

AB *C. cylindracea* produces at least two kinds of extracellular lipase, lipase I and lipase II. From the mutant which produces a large amt. of lipase I and a negligible amt. of lipase II, the authors obtained the lipase I cDNAs and genomic genes. Lipase I genes were classified into several types by sequencing and restriction anal. Lipase I proteins were thought to be encoded by several homologous genes. Lipase I was homologous with *Geotrichum candidum* lipases. Furthermore, pseudogenes were found with more than 90% homol. with lipase I cDNA. Although the amino acid compn. of purified lipase I was consistent with that deduced from the cDNA sequence, significant discrepancies were evident in the contents of leucine and serine. These discrepancies are due to the different genetic code used by *C. cylindracea*, in which the universal codon for leucine, CUG, is used to code for serine.

L22 ANSWER 56 OF 59 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN

ACCESSION NUMBER: 1993:421889 BIOSIS
DOCUMENT NUMBER: PREV199345069514
TITLE: Crystallization and characterization of *Candida rugosa* lipase.
AUTHOR(S): Rubin, Byron; Jamison, Penny; Harrison, David
CORPORATE SOURCE: Eastman Kodak Co., Rochester, NY 14650, USA
SOURCE: Alberghina, L. [Editor]; Schmid, R. D. [Editor]; Verger, R. [Editor]. GBF Monographs, (1991) pp. 63-66. GBF Monographs; Lipases: Structure, mechanisms and genetic engineering. Publisher: VCH Verlagsgesellschaft mbH, Postfach 10 11 61, Boschstrasse 12, D-6940 Weinheim, Germany; VCH Publishers, Inc., Suite 909, 220 East 23rd Street, New York, New York

10010, USA. Series: GBF Monographs.
Meeting Info.: CEC-GBF (Commission of the European
Communities-Gesellschaft fuer Biotechnologische Forschung;
(Society for Biotechnological Research) International
Workshop. Braunschweig, Germany. September 13-15, 1990.
ISSN: 0930-4320. ISBN: 3-527-28332-3, 1-56081-165-X.

DOCUMENT TYPE: Article
Conference; (Meeting)
LANGUAGE: English
ENTRY DATE: Entered STN: 15 Sep 1993
Last Updated on STN: 15 Sep 1993

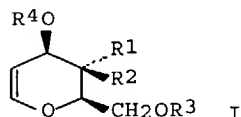
L22 ANSWER 57 OF 59 SCISEARCH COPYRIGHT (c) 2004 The Thomson Corporation.
on STN

ACCESSION NUMBER: 91:5398 SCISEARCH
THE GENUINE ARTICLE: EN927
TITLE: PURIFICATION AND SPECIFICITY OF LIPASES FROM
GEOTRICHUM-CANDIDUM
AUTHOR: BAILLARGEON M W (Reprint)
CORPORATE SOURCE: USDA ARS, EASTERN REG RES CTR, 600 E MERMAID LANE,
PHILADELPHIA, PA, 19118 (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: LIPIDS, (1990) Vol. 25, No. 12, pp. 841-848.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; AGRI
LANGUAGE: ENGLISH
REFERENCE COUNT: 31

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB A crude, commercial *Geotrichum candidum* lipase (EC 3.1.1.3)
preparation (Amano GC-20) was purified by hydrophobic interaction
chromatography on Octyl Sepharose. The purified enzyme is a
microheterogeneous glycoprotein containing isozymes varying in molecular
weight, pI and specificity. It consists of 64, 62 and 59 kDa species as
determined by denaturing polyacrylamide gel electrophoresis. Five
isozymes (pI 4.40, 4.47, 4.58, 4.67 and 4.72) are detected by isoelectric
focusing using both silver and activity stains. Chromatofocusing was used
to separate the isozymes according to pI. Although all the isozymes are
specific for oleate vs stearate esters, one isozyme (pI 4.72) is also
specific for oleate vs palmitate. The number of isozymes is reduced to
two (pI 4.67 and 4.72) after carbohydrate removal using endoglycosidase
F/N-glycosidase. These isozymes may be products of two lipase
genes.

L22 ANSWER 58 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1989:135586 CAPLUS
DOCUMENT NUMBER: 110:135586
TITLE: Enzymic synthesis of selectively protected glycals
AUTHOR(S): Holla, E. Wolfgang
CORPORATE SOURCE: Hoechst A.-G., Frankfurt/Main, D-6280/80, Fed. Rep.
Ger.
SOURCE: Angewandte Chemie (1989), 101(2), 222-3
CODEN: ANCEAD; ISSN: 0044-8249
DOCUMENT TYPE: Journal
LANGUAGE: German
OTHER SOURCE(S): CASREACT 110:135586
GI



AB Glycols I (R1 = OH, R2-R4 = H) were acylated by MeCO2CH:CH2 in the

presence of *Candida cylindracea* lipase to gene I (R1 = OH, R2 = R4 = H, R3 = Ac) while in the presence of *Pseudomonas fluorescens* lipase P I (R1 = OH, R2 = R3 = R4 = Ac) resulted. Acylation of I (R1 = OH, R2 = R4 = H, R3 = Ac, Bz) by PhCO₂CH:CH₂ in the presence of lipase P gave I (R1 = OH, R2 = H, R3 = Ac, Bz, R4 = ClCH₂CO), resp.

L22 ANSWER 59 OF 59 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1990:31430 CAPLUS

DOCUMENT NUMBER: 112:31430

TITLE: The codon CUG is read as serine in an asporogenic yeast *Candida cylindracea*

AUTHOR(S): Kawaguchi, Yoshiyuki; Honda, Hiroshi; Taniguchi-Morimura, Junko; Iwasaki, Shinjiro

CORPORATE SOURCE: Tokyo Res. Lab., Meito Sangyo Co. Ltd., Hachioji, 192, Japan

SOURCE: Nature (London, United Kingdom) (1989), 341(6238), 164-6

CODEN: NATUAS; ISSN: 0028-0836

DOCUMENT TYPE: Journal

LANGUAGE: English

AB An example of divergence from the universal genetic code is reported for the asporogenous yeast *C. cylindracea*. The nucleotide and encoded peptide sequences of a cDNA for lipase I was detd. The deduced amino acid sequence presumes that the universal codon for leucine, CUG, encodes serine. This conclusion is based on the observations that: (1) the amino-acid compn. and the partial amino-acid sequences of an extracellular lipase from this yeast agreed with those deduced from the cDNA if CUG was assumed to specify serine; and (2) serine, but not leucine, was incorporated into a polypeptide in a cell-free translation system from this yeast in the presence of a synthetic CUG oligomer.

=> file reg

=> s candida rugosa/bi

L24 51 CANDIDA RUGOSA/BI

=> d 51

L24 ANSWER 51 OF 51 REGISTRY COPYRIGHT 2004 ACS on STN

RN 124566-17-2 REGISTRY

CN Lipase, triacylglycerol (*Candida cylindracea* clone .lambda.CL115 reduced) (9CI) (CA INDEX NAME)

OTHER NAMES:

CN Lipase IA (*Candida rugosa* reduced)

FS PROTEIN SEQUENCE

MF Unspecified

CI MAN

SR CA

LC STN Files: CA, CAPLUS, CASREACT

DT.CA Caplus document type: Journal

RL.NP Roles from non-patents: BIOL (Biological study); PRP (Properties); RACT (Reactant or reagent); USES (Uses)

=> log y